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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : G01N 33/543, B01L 11/00 C12Q 1/68

A1

(11) International Publication Number: WO 92/05443

(43) International Publication Date: 2 April 1992 (02.04.92)

(21) International Application Number: PCT/GB91/01548

(22) International Filing Date: 11 September 1991 (11.09.91)

(30) Priority data:
9020216.9 15 September 1990 (15.09.90) GB
9110281.4 13 May 1991 (13.05.91) GB

(71) Applicant *(for all designated States except US):* MEDICAL RESEARCH COUNCIL [GB/GB]; 20 Park Crescent, London W1N 4AL (GB).

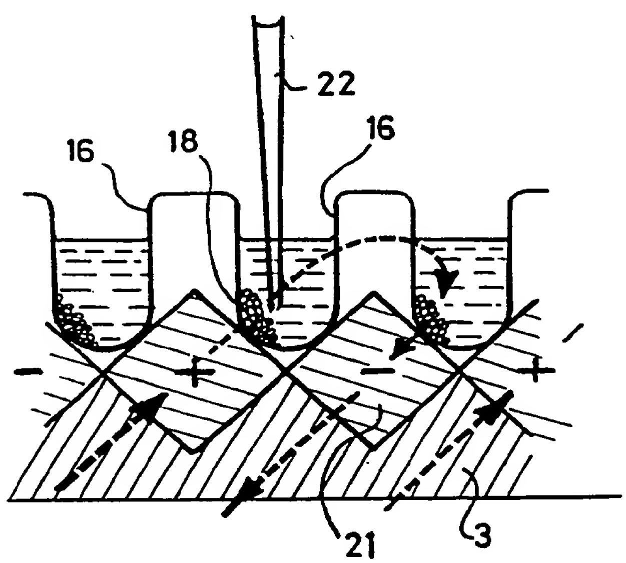
(72) Inventor; and
(75) Inventor/Applicant *(for US only)* : SCHOFIELD, Julian, Paul [GB/GB]; 19 Windsor Close, St. Ives, Cambridgeshire PE17 6DW (GB).

(74) Agent: KEITH W. NASH & CO.; Pearl Assurance House, 90-92 Regent Street, Cambridge CB2 1DP (GB).

(81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US.

Published
With international search report.

(54) Title: REAGENT SEPARATION



(57) Abstract

Apparatus for separating a target reagent attached to a solid phase magnetic support from a mixture of reagents comprises a support means (1, 13) for supporting an array of reaction vessels (16), and an associated array of permanent magnets (4, 21) arranged such that, when an array of reaction vessels is supported in the support means and the apparatus is in use for separating a target reagent from a mixture of reagents in one or more of the reaction vessels, each magnet is positioned so as to be able to exert a magnetic force on an associated reaction vessel or vessels in the array of reaction vessels, which magnetic force serves to hold the magnetic support and attached target reagent in a fixed position relative to the magnet, thus allowing the remaining mixture of reagents to be removed from the reaction vessel. The apparatus is of particular use in the preparation of DNA products for use in DNA sequencing reactions.

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Title: Reagent Separation

Field of the Invention

This invention relates to an apparatus and method for the separation of a reagent attached to a solid phase magnetic support from a mixture of reagents, which method and apparatus are of particular use in the preparation of DNA products for use in DNA sequencing reactions.

Background to the Invention

In many types of chemical and biochemical reaction, particularly those involving the production of polymers such as polypeptides or DNA molecules, reagents may be "harnessed" to a solid phase support during the reaction, so as to increase ease of identification and separation of the desired end product of the reaction.

One common type of support used to immobilise reagents in this way is a magnetic support, which may take the form of a "bead" comprising a core of a magnetic material, surrounded by a coating of a composition which will bind readily, but selectively, to the reagent to be supported. These support beads, with the reagent or product of interest still attached, can easily be separated from a reaction mixture by applying an attractive magnetic force in the vicinity of the mixture. Only magnetic support beads and attached reagents will be attracted by the magnetic force, which can be used to hold the support

beads in place whilst unwanted reagents are removed from the mixture.

One field in which such magnetic support beads are often used is that of tissue typing or immunodiagnostic assays, for which the beads are coated with a suitable antibody which will bind specifically to the target product.

Another field in which magnetic support beads are commonly used is that of the production of DNA fragments for use in DNA sequencing reactions. Each fragment to be used to sequence a DNA molecule must first be produced in sufficient quantities using a standard reaction such as the polymerase chain reaction (PCR). Relatively large quantities of the pure DNA fragments are needed if sequencing is to be carried out on a practical scale, and hence the fragments are often grown on solid phase supports in the reaction mixture, to facilitate their later separation from the mixture.

The technique of PCR relies on the use of "primers" to "copy" a base sequence of interest from a DNA reagent. These primers comprise short pieces of synthetic DNA which match (ie are complementary to) the DNA sequences at either end of the target base sequence in the DNA reagent. Heat is used to separate the two strands of the double-stranded DNA reagent. On cooling, the primer oligonucleotides then bind to their complementary sequences on the resultant DNA single strands. A suitable enzyme (a polymerase) is then added to the reaction, and acts to join nucleotides to form a new strand of DNA attached to each primer. The sequence of that new strand is complementary to that of the original DNA strand to which the primer is bound.

The reaction mixture is heated again to separate the reagents into single strand DNA, resulting in the production of twice as many target DNA strands as were originally present. The heating, cooling and polymerisation cycle is then repeated, to produce yet more copies of the target sequence attached to primers, as many times as is required. A short number of repetitions of the cycle can result in the synthesis of a large number of "copies" of the target, since each cycle results in a two-fold increase of the number of copies present.

The primers used may be labelled with a suitable label material at the 5' end. Magnetic support beads, coated with a substance which will bind chemically to the primer label, are added to the reaction mixture, and these act as a support on which the labelled primer and target DNA fragment are immobilised.

Separation of the immobilised DNA target sequences is effected using a permanent magnet. However, at present this must be done manually, since there is no apparatus available which will automatically apply the required magnetic field to a reaction mixture.

Generally, it is practical to carry out a large number of PCR reactions simultaneously (for instance, it is common to use a microtiter plate having 96 separate reaction wells, in each of which one PCR reaction is carried out). It is an extremely time-consuming process to have to manually separate the target DNA fragment from each of a large number of reaction vessels (which is generally done by holding a small permanent magnet in the vicinity of the reaction vessel whilst simultaneously removing, using a

small pipette, unwanted reaction mixture).

Furthermore, on addition of magnetic support beads to a reaction mixture in a vessel, that vessel is at present shaken manually so as to effect proper mixing of the support beads with the reaction mixture and to ensure that as much of the target DNA fragment as possible binds to the support beads. This can also be a very time-consuming process.

It is an aim of the present invention to provide a method and apparatus for separating products, such as these target DNA fragments, which have been attached to a solid phase magnetic support, from a reaction mixture, which overcome or at least mitigate the above described problems and facilitate the automation of complex reactions, in particular DNA sequencing reactions.

Statement of the Invention

According to a first aspect of the present invention there is provided apparatus for separating a target reagent attached to a solid phase magnetic support from a mixture of reagents, comprising a support means for supporting an array of reaction vessels, and an associated array of permanent magnets arranged such that, when an array of reaction vessels is supported in the support means and the apparatus is in use for separating a target reagent from a mixture of reagents in one of the reaction vessels, each magnet is positioned so as to be able to exert a magnetic force on a corresponding reaction vessel in the array of reaction vessels, which magnetic force serves to hold the magnetic support and attached target reagent in a fixed position relative to the magnet, thus allowing the

remaining mixture of reagents to be removed from the reaction vessel.

The array of permanent magnets is peferably movable between a first and a second position relative to the support means and any reaction vessels supported therein, such that when the array of magnets is in its first position, reaction vessels supported in the support means are within the influence of the magnetic field supplied by the magnets, and when the array is in its second position, the reaction vessels are outside of the influence of that field.

The array may be movable between its first and second positions by either mechanical or electrical (for instance, using an electric motor) means, and either manually or under automatic control.

The array of permanent magnets may be arranged in the same pattern as an array of reaction vessels to be supported in the support means. Each magnet then applies a magnetic field to one individual reaction vessel, which has been found to be a far more effective way of applying a magnetic force to reaction vessels than to use a single, larger, magnet to exert a magnetic force over an array of reaction vessels.

Individual permanent magnets are commercially available and in the apparatus of the present invention, each permanent magnet in the array is preferably a cylindrical magnet of depth approximately 10mm. These magnets should be arranged in the array according to the manufacturer's instructions, so that individual magnets in the array do not serve to cancel out the magnetic fields of other

magnets in the array.

Each magnet is conveniently located in an appropriately sized well in an array of wells, which array is preferably positioned below the support means. Desirably, each magnet is shaped at its end located nearest to the support means, such that reaction vessels supported in the support means can sit flush with the upper surfaces of the magnets.

In an alternative, preferred, version of the apparatus, the array of permanent magnets comprises a series of bar magnets (for instance, ferrite bar magnets) , each of which applies a magnetic field to a row of reaction vessels in an array supported in the support means. This arrangement is generally cheaper and simpler to produce.

The bar magnets are preferably arranged so as to have alternating polarities (ie adjacent magnets in the series present different polarities to reaction vessels supported in the apparatus), which increases the overall magnetic field which the array of magnets is capable of exerting.

The magnets in the array, in both versions of the apparatus described above, are preferably arranged at such an angle to reaction vessels supported in the support means that the magnetic support and attached target reagent in any reaction vessel can be held, by the magnetic force exerted by the magnets, to one side of the vessel. The advantage of holding the magnetic support to one side of, rather than directly at the bottom of, the reaction vessel is that it is easier, when adding reagents to or removing them from the vessel, (for instance by pipetting) to avoid inadvertently removing any of the

magnetic support and attached target reagent.

The support means is preferably of such dimensions as to accommodate a standard 96-well microtiter plate, in which DNA sequencing and other reactions are commonly carried out. In this case, the array of permanent magnets may, for instance, comprise 96 magnets arranged in 8 rows of 12, the position of each magnet corresponding to the position of one of the 96 wells in the microtiter plate. Alternatively, the array may comprise a series of, for example, nine bar magnets arranged with their longitudinal axes parallel, each bar positioned so as to be able to apply a magnetic field to one of the 8 rows of 12 wells in the microtiter place.

The present invention also provides apparatus as described above, in combination with an array of reaction vessels supported in the support means.

The apparatus may also include agitating means for agitating reaction vessels supported in the apparatus, so as to facilitate mixing of reagents contained in the reaction vessels. Hence, according to a further aspect of the invention, there is provided apparatus for separating a target reagent attached to a solid phase magnetic support from a mixture of reagents, comprising a support means for supporting an array of reaction vessels; agitating means for agitating reaction vessels supported in the apparatus; and an associated array of permanent magnets arranged such that, when an array of reaction vessels is supported in the support means and the apparatus is in use for separating a target reagent from a mixture of reagent in one of the reaction vessels, each magnet is positioned so as to be able to exert a magnetic

force on a corresponding reaction vessel in the array of reaction vessels, which magnetic force serves to hold the magnetic support and attached target reagent in a fixed position relative to the magnet, thus allowing the remaining mixture of reagents to be removed from the reaction vessel.

Any suitable agitating means may be used, for instance, electrically vibrating crystals may be provided for use inside each reaction vessel - this allows for the apparatus of the present invention to be extremely compact in size. The agitating means may, however, be mechanical in operation, and may comprise, for instance, an orbital shaker of conventional construction, which is operational to move the reaction vessels around in the horizontal plane.

The agitating means of the apparatus is preferably automatically rendered non-operational whenever the array of magnets is in its first position relative to the support means. This ensures that reaction vessels supported in the apparatus are not agitated at a time when any magnetic support contained in the vessels is fixed in position by the magnetic field of the permanent magnets, and hence reduces the risk of spillage of reagents not attached to the magnetic support.

The apparatus may additionally comprise control means for controlling the speed of agitation of the reaction vessels. The agitating means may be rendered automatically non-operational when a certain pre-determined speed of shaking is reached - such an upper "cut-off" speed is also for safety purposes and to prevent spillage of reagents. This is particularly important

since reagents spilled from one reaction vessel may actually contaminate reactions carried out in other vessels in the array, or future reactions carried out in the same apparatus.

Additionally, the apparatus may comprise timer means by which the user may control the agitating means to be operational for a desired period of time.

The apparatus may additionally comprise reagent removal means by which unwanted reagents, not attached to the magnetic support, may be removed from reaction vessels supported in the apparatus. Such reagent removal means may comprise, for instance, a robotic work-arm set up to aliquot quantities of unwanted reagents from the reaction vessels.

The apparatus of the present invention may form part of a complete automatic DNA sequencing machine, and the present invention accordingly provides DNA sequencing apparatus comprising separation apparatus for separating a target DNA fragment attached to a solid phase magnetic support from a mixture of reagents, the separation apparatus being as described above as being in accordance with the present invention.

According to yet a further aspect of the present invention, there is provided a method of separating a target reagent attached to a solid phase magnetic support from a mixture of reagents, comprising the steps of placing a reaction vessel containing the mixture of reagents in the support means of apparatus in accordance with the invention; allowing a magnet in the array of permanent magnets of the apparatus to exert a magnetic

force on the reaction vessel so as to hold the magnetic support and attached target reagent in a fixed position relative to the magnet; and removing unwanted reagents from the reaction vessel whilst the magnetic support is so held in position.

Following these steps, the target reagent can be separated from the magnetic support in any conventional manner. The method preferably additionally comprises steps of washing the magnetic support and attached target reagent, using an inert washing agent, following removal of unwanted reagents, and repeating the separation process using the permanent magnet of the apparatus.

The target reagent may be a labelled DNA fragment, in which case the fragment is preferably labelled with a 5' biotin label and the magnetic support comprises a magnetic bead having a streptavidin or avidin coating.

Alternatively, the mixture of reagents may be for use in tissue typing or immunodiagnostic assays, in which case the magnetic supports will typically comprise a magnetic bead having a coating of an appropriate antibody.

The reaction vessel is preferably supported in apparatus comprising agitating means, and the method then additionally comprises the step of agitating the reaction vessel so as to facilitate binding of the magnetic support to the target reagent.

The apparatus and method of the present invention will now be described in more detail, by way of example only, with reference to the accompanying drawings, of which:-

Figure 1 is a cross-section of apparatus in accordance with the present invention;

Figure 2 is an exploded perspective view of part of Figure 1;

Figure 3 shows schematically a reaction vessel supported in the apparatus of Figures 1 and 2;

Figure 4 is a schematic plan view showing an alternative arrangement of the magnets in the apparatus of Figures 1 and 2;

Figure 5 shows schematically a reaction vessel supported in the apparatus of Figures 1 and 2 having the alternative magnet arrangement of Figure 4; and

Figure 6 illustrates a scheme for a typical PCR DNA synthesis using a magnetic support to assist in isolation of the target fragment.

Detailed Description of the Drawings

The apparatus shown in Figure 1 comprises support means which in turn comprises an upper (1) and a lower (13) support plate, separated by four aluminium alloy columns 14. The upper plate 1 is constructed from a polycarbonate material and has a rectangular recess 2 (see Figure 2) to accommodate a standard 96-well microtiter plate.

Lower supporting plate 13 is constructed from aluminium alloy.

Also mounted in the apparatus is a polycarbonate plate 5,

to which is securely attached a magnet block 3 which comprises a nylon polymer block in which 96 permanent magnets 4 (not shown in Figure 1) are mounted in a 8x12 array. Spring loaded pillars 6 pass through four holes, one at each corner of plate 5, connecting the plate to a scissor-type jack mechanism 7, which serves to raise and lower plate 5 and magnet block 3 relative to a microtiter plate supported by support plate 1. The magnet block 3 can thereby be moved between a first and a second position. When in its first position, the magnet block exerts a magnetic force on reaction vessels supported in plate 1; when the block is in its second position (shown in Figure 1), any supported reaction vessels are outside of the influence of that magnetic force.

Operation of jack mechanism 7 is controlled by means of a motor leadscrew assembly 9, which is connected to the jack mechanism by moving connector 8 and powered by a mains driven power supply 10. Power supply 10 is also equipped with on-off switches and other controls (not shown). It is, however, quite feasible for the magnet block 3 to be moveable between its first and second positions by purely mechanical means, if simplicity or speed of operation are to be increased or apparatus production costs to be reduced.

Component 11 is an orbital shaker, such as is commercially available, to which the support plates 1 and 13 are securely attached. This is also driven by the motor assembly 9.

A microswitch 12 ensures that the shaker 11 is automatically switched off when the magnet block 3 is raised to its first position.

Figure 2 shows more clearly the upper support plate 1 with its recess 2, support columns 14 and lower support plate 13. Part of the array of permanent magnets 4 in the magnet block 3 is also visible. Circular holes 28 in plate 5 are those through which the spring-loaded pillars 6 (shown in Figure 1) pass and together with a pair of elongate holes 29, for location of cones 15 on plate 1, these form a floating friction system to allow for alignment of the magnet block 3 with a microtiter plate supported in recess 2. Alignment must be such that each magnet 4 in the block 3 sits directly below one of the 96 wells in a standard microtiter plate supported in recess 2. The locating holes 29 are elongate in shape so as to allow for movement of the support plates in the horizontal plane when the orbital shaker 11 is operational.

Figure 3 shows schematically how each well of a microtiter plate supported in recess 2 is positioned relative to the magnets 4 when the block 3 has been raised to its first position. Each well 16 of the microtiter plate constitutes a reaction vessel, which may contain, for instance, a reagent mixture 19 and magnetic support beads 18 to which the target reagent is attached. Each permanent magnet 4 is located in a small well 17 in magnet block 3, and has a concave upper end so that the reaction vessel 16 can sit flush with the top of the magnet. In this position, the magnetic support beads 18 are held in a fixed position under the influence of the magnet 4, and unwanted reagents can be removed from vessel 16, either manually, using a standard "Gilson Tip" pipette, or by means of a robotic arm which may be incorporated in the apparatus.

The magnet block 3 may alternatively have the arrangement shown schematically in Figure 4. Here, a standard 96-well microtiter plate 20 is shown in plan view, as though supported in the apparatus of Figures 1 and 2 (not shown). The magnet block supported below the plate 20 comprises nine ferrite bar magnets 21, each of 36 mm^2 square cross section, each of which is positioned between two of the rows A-H of wells in the plate 20 and each of which serves to exert a magnetic force on at least one of these rows of wells.

The bar magnets 21 are arranged, by means of a suitably shaped support block 3, at an angle to the wells 16 of plate 20, as shown in Figure 5. A magnetic support, such as beads 18, in a well 16, is therefore held by the magnetic force exerted by bars 21 to one side of the well rather than directly at the bottom, the beads being drawn towards the user of the apparatus. Reagents can thus be added to or removed from the wells, for instance using pipette 22, with a reduced risk of inadvertently removing beads 18 and any attached target reagent.

Figure 5 also shows how the bar magnets 21 are arranged to have alternating polarities (+,-), which increases the overall strength of the magnetic field exerted by the magnets. The magnetic field is indicated by the dotted lines.

One method of use of the apparatus shown in Figures 1-5 is to separate target DNA fragments, attached to magnetic support beads, from a reagent mixture for subsequent use in DNA sequencing reactions. The target DNA fragment might typically be generated by the PCR technique, which is illustrated schematically in Figure 6. A sample of

double-stranded DNA, containing the fragment desired to be reproduced, comprises the complementary strands 23 and 24. Two primers 25 and 26 are used to build up "copies" of the desired fragment from the separated strands 24 and 25 respectively (Figure 6A) to result in the incorporation of a large number of primers 25 into the target DNA fragments copied from strand 24 (Figure 6B).

Primer 25 has been labelled with a biotin label 27 and, following the PCR reaction (which is carried out in one well of a microtiter plate), between 15 and 30 ul of (previously washed) streptavidin-coated magnetic beads 28 are added to the reaction well (Figure 6B). An example of such a support bead is shown in cross-section in Figure 6D - the bead comprises a ferric magnetic core 29 and a streptavidin (to which biotin can bind chemically) shell 30. These beads appear to be liquid in nature when added to a reaction mixture, but under the influence of a magnetic field, take on the appearance of individual beads.

At this stage, the microtiter plate, in which any number of such reactions (up to a maximum of 96) may be carried out, should be positioned in recess 2 of the support block 1 of the apparatus. Retaining clips, not shown in the Figures, are used to hold the microtiter plate in position. The user switches on the "mixing" function of the apparatus, thus bringing orbital shaker 11 into operation to agitate the reaction wells in the mictotiter plate. This serves to mix reagents contained in the wells, and thus to ensure efficient binding of the support beads 28 to the labelled primers 25. Agitation should be carried out for a period of between 5 and 10 seconds.

The procedure is then as follows:-

1. Allow the reaction mixtures to stand for 15 minutes to allow for complete binding of the support beads and the primer.

2. Add 20 ul of 0.45M NaOH to the reaction mixtures and agitate for 5-10 seconds using the orbital shaker of the apparatus. Addition of this alkali serves to separate the strands, corresponding to strands 23 and 24, of each DNA fragment. The reaction mixture should be left to stand for 5 minutes at room temperature to allow complete separation to occur. This will result in the reaction mixture containing biotinylated single strands of the target DNA fragment and separate, unlabelled, complementary single strands of (unwanted) DNA (Figure 6C).

3. The user of the apparatus now switches on the jack mechanism 7 to raise the magnet block 3 towards the microtiter plate so as to bring the reaction wells within the magnetic field provided by magnets 4 (or 21). The support beads 28 in the reaction wells should rapidly form into pellets (within about 15 seconds). The magnet block should be left in its raised position whilst the user carefully aspirates the unwanted reagents from each reaction well using a Gilson Tip. The magnet block should then be lowered so as to bring the reaction wells outside of the influence of its magnetic field.

4. The pellets remaining in the reaction wells are washed by adding a further 20 ul of 0.15M NaOH. The orbital shaker is again switched on so as to effect mixing of the pellets with the alkali.

5. Again, the orbital shaker is switched off and the magnets raised so as to cause pelleting of the support beads and to allow for removal of the supernatant with a Gilson Tip.

6. Following a similar procedure to that outlined in steps 1-5, the pellets are now washed three times with 40 ul of double distilled deionized water. After each washing, the supernatant is removed whilst the reaction wells remain under the influence of the magnets 4 (or 21).

This method results in the production of target DNA fragments attached to the magnetic support beads and isolated from unwanted reagents. The target DNA fragments can then be separated from the support beads using conventional techniques.

The apparatus is equipped with a microswitch 12 which ensures that (through its contact with the moving connector 8) the orbital shaker 11 is automatically rendered non-operational when the magnet block 3 is raised so as to exert a magnetic field on reaction vessels supported by plate 1. This ensures that the user of the apparatus cannot accidentally continue to agitate the reaction vessels whilst they are under the influence of the magnets, and thus reduces the risk of reagent spillage.

It will be clear from the above that the apparatus and method of the present invention allow for the controlled mixing of reagents and, more particularly, for separation of reagents attached to magnetic supports from reagent

mixtures, with an ease and controllability not previously available. The magnetic source is custom-built for use in cases where a large number of reactions are carried out simultaneously (for instance, in a standard microtiter plate) and can be brought into and out of the reaction area with ease. The apparatus also lends itself to automation and to incorporation into other, larger scale, sequencing apparatus.

Claims

1. Apparatus for separating a target reagent attached to a solid phase magnetic support from a mixture of reagents, comprising a support means for supporting an array of reaction vessels, and an associated array of permanent magnets arranged such that, when an array of reaction vessels is supported in the support means and the apparatus is in use for separating a target reagent from a mixture of reagents in one or more of the reaction vessels, each magnet is positioned so as to be able to exert a magnetic force on an associated reaction vessel or vessels in the array of reaction vessels, which magnetic force serves to hold the magnetic support and attached target reagent in a fixed position relative to the magnet, thus allowing the remaining mixture of reagents to be removed from the reaction vessel or vessels.

2. Apparatus according to claim 1, wherein the array of permanent magnets is movable between a first and a second position relative to the support means and any reaction vessels supported therein, such that when the array of magnets is in its first position, reaction vessels supported in the support means are within the influence of the magnetic field supplied by the magnets, and when the array is in its second position, the reaction vessels are outside of the influence of that field.

3. Apparatus according to claim 1 or 2, wherein the array of permanent magnets is arranged in the same pattern as an array of reaction vessels to be supported in the support means.

4. Apparatus according to claim 3, wherein each

permanent magnet in the array is a cylindrical magnet of depth approximately 10mm.

5. Apparatus according to claim 3 or 4, wherein each magnet is located in an appropriately sized well in an array of wells, which array is positioned below the support means.

6. Apparatus according to claim 1 or 2, wherein the array of permanent magnets comprises a series of bar magnets, each of which applies a magnetic field to a row of reaction vessels in an array supported in the support means.

7. Apparatus according to claim 6, wherein the bar magnets are arranged so as to have alternating polarities, with adjacent magnets in the series presenting different polarities to reaction vessels supported in the apparatus.

8. Apparatus according to anyone of the preceding claims, wherein the magnets in the array are arranged at such an angle to reaction vessels supported in the support means that the magnetic support and attached target reagent in any reaction vessel can be held, by the magnetic force exerted by the magnets, to one side of the vessel.

9. Apparatus according to any one of the preceding claims, wherein the support means is of such dimensions as to accommodate a standard 96-well microtiter plate.

10. Apparatus according to any one of the preceding claims, in combination with an array of reaction vessels

supported in the support means.

11. Apparatus according to any one of the preceding claims, further comprising agitating means for agitating reaction vessels supported in the apparatus.

12. Apparatus according to claim 11, wherein the agitating means comprise electrically vibrating crystals for use inside each reaction vessel.

13. Apparatus according to claim 11, wherein the agitating means comprise an orbital shaker.

14. Apparatus according to claim 11, 12 or 13, when dependent on claim 2, wherein the agitating means are automatically rendered nonoperational when the array of magnets is in its first position relative to the support means.

15. Apparatus according to any one of claims 11 to 14, further comprising control means for controlling the speed of agitation of the reaction vessels.

16. Apparatus according to claim 15, wherein the agitating means are automatically rendered non-operational when a predetermined speed of shaking is reached.

17. Apparatus according to any one of claims 11 to 16, further comprising timer means for controlling the period of operation of the agitating means.

18. Apparatus according to any one of the preceding claims, further comprising reagent removal means for removing reagents, not attached to the magnetic support,

from reaction vessels supported in the apparatus.

19. DNA sequencing apparatus comprising separation apparatus in accordance with any one of the preceding claims for separating a target DNA fragment attached to a solid phase magnetic support from a mixture of reagents.

20. A method of separating a target reagent attached to a solid phase magnetic support from a mixture of reagents, comprising the steps of placing a reaction vessel containing the mixture of reagents in the support means of apparatus in accordance with any one of the preceding claims; allowing a magnet in the array of permanent magnets of the apparatus to exert a magnetic force on the reaction vessel so as to hold the magnetic support and attached target reagent in a fixed position relative to the magnet; and removing unwanted reagents from the reaction vessel whilst the magnetic support is so held in position.

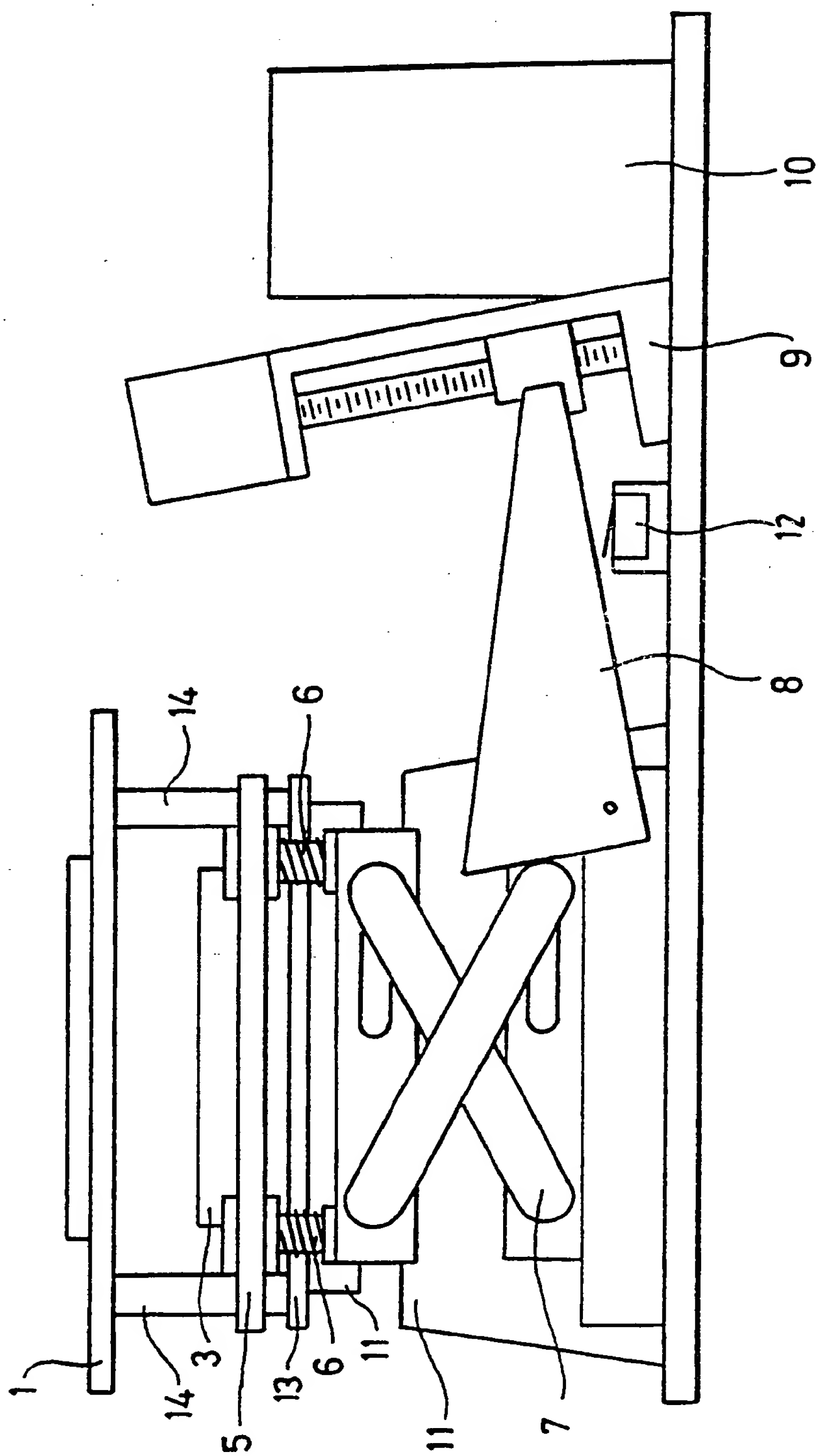

Fig. 1

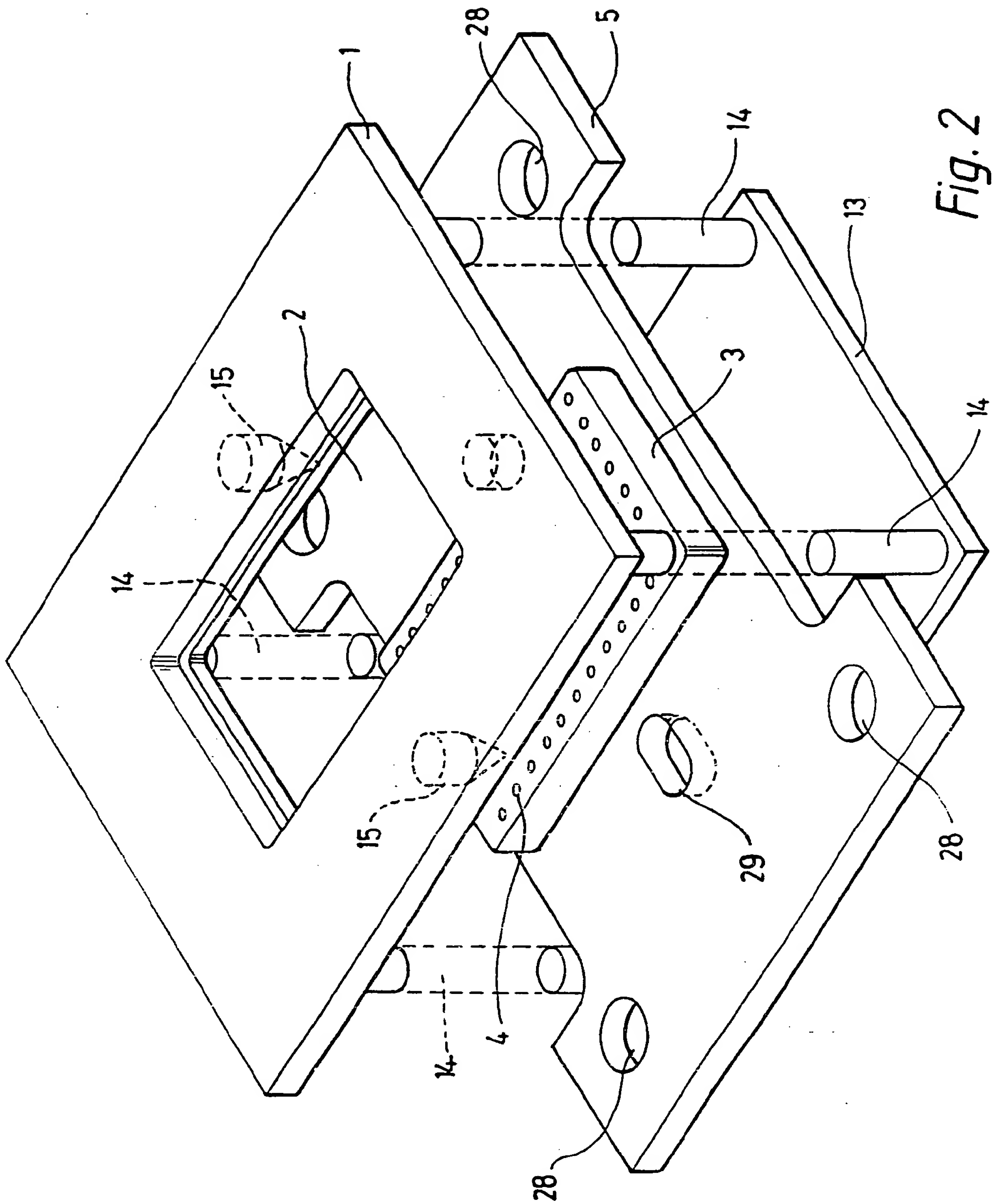

Fig. 2

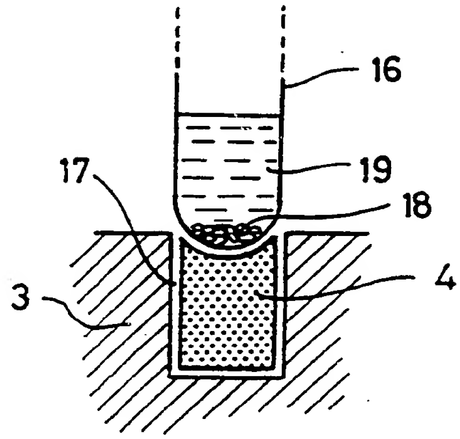

Fig. 3

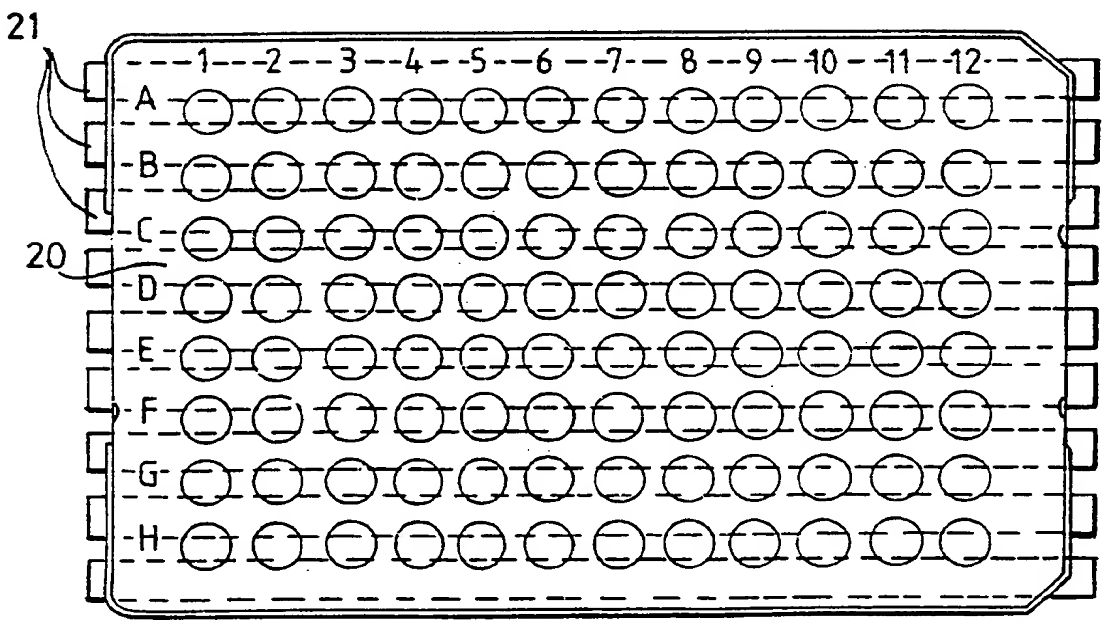

Fig 4

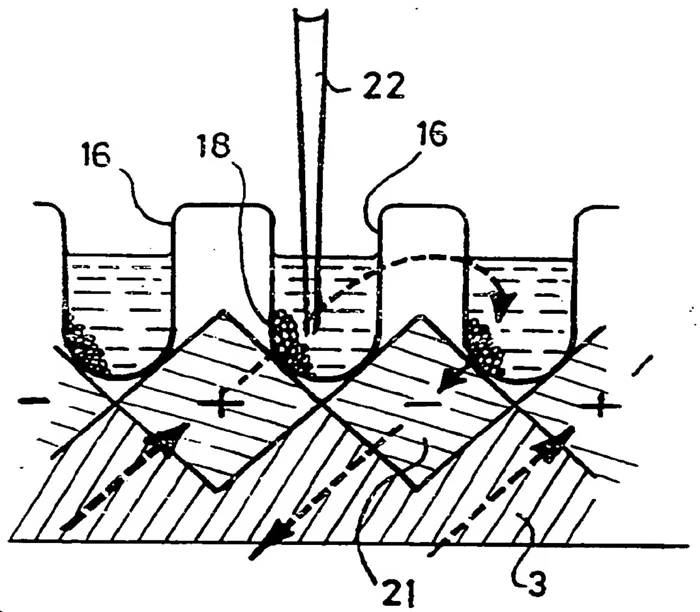

Fig. 5

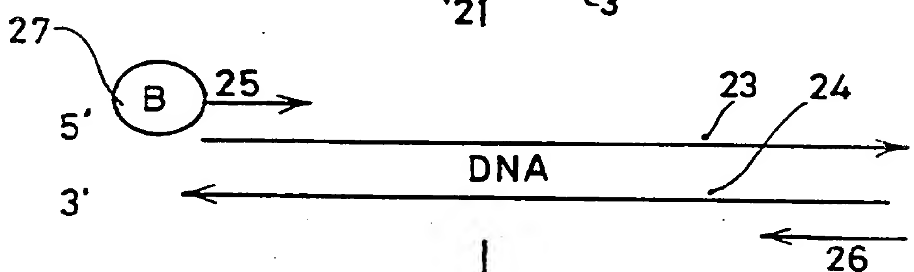

Fig.6A

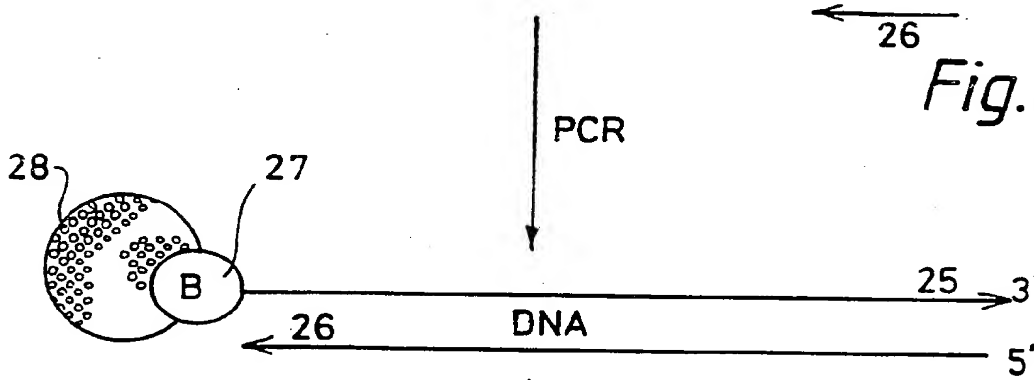

Fig.6B

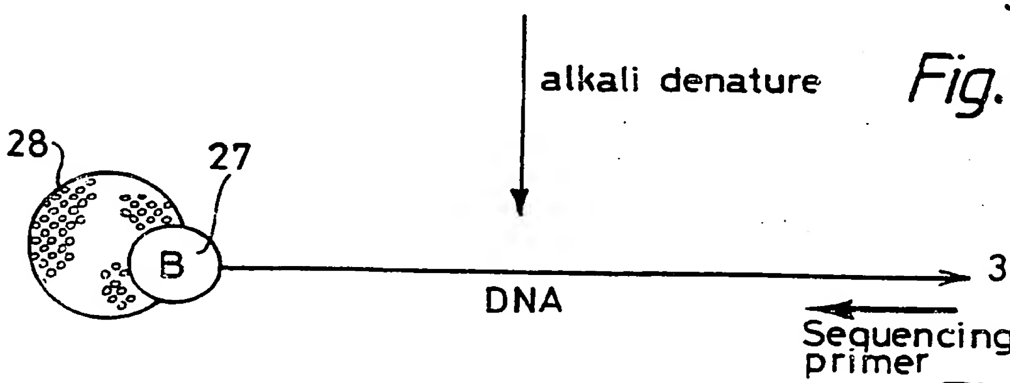

Fig.6C

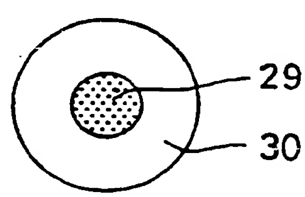

Fig. 6D

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 91/01548

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 G01N33/543; B01L11/00; C12Q1/68

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	B01L ; G01N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X	EP,A,0 317 286 (GENE-TRAK SYSTEMS) 24 May 1989	1-3,5, 8-10, 18-20
	see column 3, line 3 - line 60 see column 5, line 21 - column 8, line 2; figures 1-4; example 1	
A		11

X	EP,A,0 355 823 (E.I. DU PONT DE NEMOURS & CO.) 28 February 1990	1-3,8, 10,11, 17,18,20
	see page 5, line 38 - page 6, line 31; figures 3,4	

X	EP,A,0 030 086 (TECHNICON INSTRUMENTS COMPANY LTD.) 10 June 1981 see page 11, line 20 - line 30; figure 2 see page 12, line 23 - line 30; figure 7	1-3,5,6

	-/--	

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
09 DECEMBER 1991	17. 01. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	HODSON C.M.

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International Application No PCT/GB 91/01548

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	WO,A,9 006 045 (DYNAL A.S.) 14 June 1990 see page 1, line 1 - page 3, line 20 ---	1,19,20
A	EP,A,0 351 857 (OLYMPUS OPTICAL CO. LTD.) 24 January 1990 ---	
A	WO,A,8 901 161 (INT. INST. OF CELLULAR & MOLECULAR PATHOLOGY) 9 February 1989 ---	

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9101548 SA 51139

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 09/12/91
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Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0317286	24-05-89	AU-A-	2561988	18-05-89
		JP-A-	1201156	14-08-89
		US-A-	4988618	29-01-91
EP-A-0355823	28-02-90	JP-A-	2269970	05-11-90
EP-A-0030086	10-06-81	CA-A-	1152559	23-08-83
		US-A-	4438068	20-03-84
WO-A-9006045	14-06-90	AU-A-	4758690	26-06-90
		EP-A-	0446260	18-09-91
		AU-A-	4758590	26-06-90
		WO-A-	9006044	14-06-90
		EP-A-	0444119	04-09-91
		AU-A-	4759690	26-06-90
		WO-A-	9006042	14-06-90
		EP-A-	0444120	04-09-91
		AU-A-	4666489	26-06-90
		WO-A-	9006043	14-06-90
		EP-A-	0448609	02-10-91
EP-A-0351857	24-01-90	JP-A-	2107968	19-04-90
		JP-A-	2124464	11-05-90
WO-A-8901161	09-02-89	None		

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For more details about this annex : see Official Journal of the European Patent Office, No. 12/82